Original Article

The value of qualitative detection of human chorionic gonadotropin in vaginal washing fluid for diagnosis of preterm premature rupture of membranes

Farideh Movahed[1], Khadijeh Elmizadeh[2], Amir Javadi[3], Akram Choopani[4]

ABSTRACT

Objective: This study was performed to determine the sensitivity and specificity of qualitative hCG testing of vaginal washings for diagnosis of preterm premature rupture of membranes (PPROM).

Methodology: A total of 92 singleton pregnant women at 20 – 37 weeks of gestation were placed in two equal groups of documented rupture of membranes and intact membranes as control. Confirmatory tests included pooling of amniotic fluid in the vaginal vault with positive ferning test. Patients with vaginal bleeding and obvious fetal anomaly were excluded. After cleaning the vagina with a sterile tampon and irrigation with 5 ml sterile saline, fluids were collected from the posterior vaginal fornix. Samples were tested by a pregnancy test strip with sensitivity of 25 mIU/ml. Data were collected and analyzed using t-test, Mann-Whitney, Chi-squared, Fisher's exact tests.

Results: The groups had similar demographic characteristics. The hCG test was positive in 38 (83%) of the ruptured membranes group and in 4 (8%) of the control group. Sensitivity, specificity, positive predictive value, and negative predictive value were shown to be 83%, 91%, 90%, and 84%, respectively.

Conclusions: The qualitative determination of hCG in vaginal washings proved a simple, rapid, noninvasive, and reliable test in the diagnosis of PPROM.

KEY WORDS: Chemical biomarkers, Premature rupture of membranes, Qualitative hCG test.

Pak J Med Sci October - December 2012 Vol. 28 No. 5 900-903

How to cite this article:

Movahed F, Elmizadeh K, Javadi A, Choopani A. The value of qualitative detection of human chorionic gonadotropin in vaginal washing fluid for diagnosis of preterm premature rupture of membranes. Pak J Med Sci 2012;28(5):900-903

1. Farideh Movahed,
Obstetrician and Gynecologist,
Dept. of Obstetrics & Gynecology,
2. Khadijeh Elmizadeh,
Obstetrician and Gynecologist,
Dept. of Obstetrics & Gynecology,
3. Amir Javadi,
Instructor of Biostatistics,
Dept. of Biostatistics,
4. Akram Choopani,
Resident of Obstetrics and Gynecology,
Dept. of Obstetrics & Gynecology,
1-4: Qazvin University of Medical Science, Qazvin, Iran.

Correspondence:

Khadijeh Elmizadeh,
E-mail: khelmizadeh@qums.ac.ir
parisa_elmizadeh@yahoo.com

* Received for Publication: March 24, 2012
* Revision Received: August 16, 2012
* Revision Accepted: August 18, 2012

INTRODUCTION

Preterm labor occurs in approximately 11% of all deliveries, and preterm premature rupture of membranes (PPROM) is the causing factor in 30 – 35% of cases.[1] Despite recent advances in prenatal care, PPROM continues to lead to serious fetal and maternal morbidity. Some complications such as infection, prematurity, and cord prolaps are associated with the PPROM itself, and the others including failure of labor induction and prolonged labor are due to clinical management.[2] Thus, the accurate diagnosis of PPROM is crucial.

Diagnosis of rupture of membranes is easy when there is a visible leakage of amniotic fluid from the cervix, but it is more difficult when leakage cannot

be documented. Various tests have been used for confirming PPROM. Patient's history, ferning pattern after microscopic examination, and the nitrazin test all are helpful but have been criticized for their high rate of false positive and false negative results. Nitrazin test is associated with a high false positive rate (17.4%), which is related to cervicitis, vaginitis, and contamination with blood, urine, semen, and antiseptic agents. The ferning test may give false positive results (5.8%) due to fingerprints or contamination with semen and cervical mucus.[3,4] False negative rates of 9.4% for nitrazine test and 12.9% for ferning have been reported.[5]

Because of the potential limitations mentioned above, several biochemical markers in vaginal fluid have been evaluated for detection of PPROM: aminotransferase, prolactin, alpha-fetoprotein, fetal fibronectin, insulin-like growth factor binding protein 1, and thyroid hormones. However, these efforts have been met with limited success.[6-10]

Recently, beta subunit of human chorionic gonadotropin (β-hCG) has been evaluated as a marker for diagnosis of PPROM. HCG is produced by the trophoblastic tissue and is present in varying degrees in maternal serum, urine, and amniotic fluid during pregnancy. In a review of previous research, we found several studies concerning quantitative measurement of hCG in cervicovaginal fluids. Investigators in Japan, Italy, China, and Turkey have established different cut-off points of β-hCG (6-100mIU/ml) for diagnosis of PPROM in cervicovaginal secretions.[11-14] Esim et al. in their study showed a sensitivity of 86%, specificity of 93%, PPV of 67%, and NPV of 97% for quantitative hCG testing of cervicovaginal washing in the second trimester of pregnancy.[13] Also, Ni et al. showed similar results.[12]

In contrast, a few of the studies we reviewed were concerned with qualitative hCG testing of cervicovaginal washing. For instance, Cooper et al in 2004 evaluated this method to detect PPROM for the first time and found it to be valuable in diagnosis of PPROM, with a sensitivity of 79% and a specificity of 96%.[14]

On the whole, it seems that quantitative measurement of hCG in vaginal fluids as a PPROM marker is costly and time-consuming, and that qualitative hCG testing, if proved effective, can be a good substitute. It is more rapid and cheaper and can be done at the bedside.

The present research is a study of diagnostic accuracy and was performed to determine the sensitivity and specificity of qualitative hCG testing in the diagnosis of preterm premature rupture of membranes in confirmed cases that did not have a large amount of amniotic fluid in the vagina.

METHODOLOGY

We prospectively selected patients attending obstetrics ward at Kosar hospital, a tertiary care university-affiliated center in Qazvin in Iran, from July to December 2010. Ninety-two singleton pregnant women at 20-37 weeks of gestation participated in the research. Patient selection was carried out during the times the investigator was available. The ethics committee of Qazvin University of Medical Science approved the study protocol (approval number: 28/20/4417). Written informed consent was obtained from each participant.

Cases with vaginal bleeding and obvious anomaly in their fetuses were excluded from the study. Patients were divided into two equal groups. Group I consisted of women with documented PPROM, and group II consisted of women with intact amniotic membranes as control. We inserted sterile speculum for eligible patients with or without a complaint of PPROM. Rupture of membranes was confirmed by visualized amniotic fluid pooling in posterior vaginal fornix and ferning pattern of the dried sample. The patient was considered to have intact membranes if she had no complaint of leakage, there was no fluid pooling, and we had a negative ferning test. We decided to clean and dry the vagina from secretions or extra fluid in order to simulate the suspicious status of PPROM where there is no obvious amniotic fluid in the vagina. Posterior vaginal fornix was then irrigated with 5ml sterile saline using a 5-ml syringe under direct visualization. Subsequently, the vaginal fluids were aspirated from the posterior fornix with the same syringe. Next, a one-step qualitative hCG test strip with a sensitivity of 25 IU/ml was inserted in the cervicovaginal washing for 10 seconds. After five minutes, hCG qualitative test was interpreted as positive with the appearance of two colored lines, negative if one line appeared, and invalid if no line appeared. Invalid tests were repeated with another stip.

Demographic data, gestational age, and hCG test results were recorded for each patient. Two evaluation tests of Positive Likelihood ratio (PLR) and Negative Likelihood ratio (NLR) are valuable in making clinical decisions. Thus, PLR and Confidence interval were used to calculate the sample size, which turned out to be 92. The following assumptions formed the basis of the calculation: (1)

experimental and control groups were comprised of the same number of members, (2) the lower limit of the PLR was equal to 5, and (3) the Confidence interval was 95%. According to the calculated sample size, the study would continue as long as the golden standard test confirms that 46 of the participants are patients and 46 of them are healthy. Based on this sample size, the positive likelihood ratio would be more than 5 with 95% confidence.

Data analysis was performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were summarized as either mean and standard deviation, median (minimum-maximum), or percentage where appropriate. For 2x2 tables, we computed sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and likelihood ratios. The data were analyzed using t-test, Mann-Whitney U-Test, Chi-squared, and Fisher's exact tests. Statistical significance was considered at $P<0.05$.

RESULTS

Overall, 92 patients were studied with 46 in the PPROM group and 46 in the control group. Both groups were similar with respect to maternal age, parity, and gestational age at the time of testing (Table-I).

In the PPROM group, 38 of the patients had a positive hCG test, resulting in a sensitivity of 83%. In contrast, 42 of the patients in the control group had a negative hCG test, demonstrating a specificity of 91%. Our study showed that a positive hCG test in vaginal washing had a PPV of 90% and a NPV of 84% (PLR = 9.2, NLR =0.19; P-Value<0.001). We had two invalid test results in the control group which were negative after the test was repeated with another strip.

DISCUSSION

The results indicate that qualitative hCG testing of vaginal washing is useful in detecting PPROM, with a sensitivity of 83%, specificity of 91%, PPV of 90%, and NPV of 84%. This is consistent with the findings of Cooper et al., which demonstrated a sensitivity and specificity of 79% and 96%, respectively, for the test, using Quickvue one-step pregnancy test with a threshold of 25 mIU/ml.[14] Karimian et al in their study examined vaginal washing in two ways, using a home pregnancy test with a threshold of 25 mIU/ml and through quantitative measurement of hCG[15]. For the home pregnancy test, sensitivity, specificity, PPV, and NPV were 97.7%, 88.4%, 89.4%, and 97.5%, respectively. They found a very good agreement between the results obtained from the two methods.

Table-I: Demographic data of the patients.

	PPROM (N=46)	*Control (N=46)*	*P-Value*
Age (year) *	25.7 ±5.7	26.4 ±5.7	0.57
Gestational Age (weeks) *	30.3 ±4.4	29.2 ±4.6	0.23
Parity †	0 (0-3)	0 (0-3)	0.09
Gravity †	2 (1-5)	1 (1-6)	0.08

* Mean ±SD; † Median (Minimum-Maximum)

We cleaned and dried the vagina with a sterile tampon before irrigation and aspiration. This resulted in lower concentration of amniotic fluid in the collected samples. Cooper and Karimian did not dry the vagina from secretions and extra amniotic fluid.[14,15] We expected to have less strong results, but they turned out to be similar. This can be attributed to the high concentration of hCG in amniotic fluid. As a normal pregnancy progresses, the mean level of β-hCG in maternal blood increases to approximately 54000 mIU/ml at 8-12 weeks of gestation. It then declines rapidly to around 12000 mIU/ml and remains at this level up to the end of the pregnancy. Beta hCG is also present in amniotic fluid at concentrations ranging from 2000-70000 mIU/ml. In addition, it is secreted by cervical glands and is present in the vaginal secretions. Esim et al. and Anai et al. evaluated hCG as a marker for PPROM and found vaginal fluid hCG levels of normal pregnant women to be 37.9, 9.5, and 6.3 mIU/ml during the first, second, and third trimesters, respectively.[13,16] The hCG level of women with PPROM was shown to be 420.6 mIU/ml. They concluded that the hCG level in vaginal fluid is a useful marker of PPROM during the second and third trimesters.

Given this discussion, it seems that a home pregnancy hCG strip with a threshold of 25 mIU/ml can be useful in detecting rupture of membranes, especially in the second and third trimesters when the normal level of hCG in the vagina is below this threshold. Removing extra amniotic fluid and secretions from the vagina proved the high detective sensitivity of hCG qualitative test even if there is very subtle and invisible amount of amniotic fluid.

However, in the patients with suspected rupture of membrane, confirmation of PROM may be impossible, and some of them may have intact membranes. Hence, the results of hCG test cannot be exclusively attributed to the PROM patients. To solve this problem, we tried to simulate the

condition of the suspected cases of PROM in patients with actual PROM. As a result, caution must be taken when the results are extrapolated to the actual cases of PROM.

It is important to note that this diagnostic method is only applicable in the absence of blood in the vagina. Vaginal bleeding can culminate in false positive results due to high concentration of hCG in the blood.

In conclusion, the diagnosis of rupture of membrane can be very difficult when there is only minimal amniotic fluid leakage. In this study, we demonstrated a high sensitivity, specificity, PPV, and NPV for the hCG qualitative test in cervicovaginal washing in patients with confirmed rupture of membranes but subtle and invisible amount of amniotic fluid in the vagina. These findings show that this simple, rapid, and inexpensive test may be useful in detecting PPROM in equivocal cases. Nonetheless, given the paucity of studies in this field, further research seems to be necessary to obtain more conclusive results for clinical use.

Source of funding: Qazvin University of Medical Science Qazvin, Iran.

REFERENCES

1. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. Williams Obstetrics. 23th ed., New York: McGraw-Hill; 2010: 811.
2. Svigos JM, Dodd JM, Robinson JS. Threatened and actual preterm labor including mode of delivery. In: James D, Steer PJ, Weiner CP, Gonik B, Crowther CA, Robson SC, editors. High risk pregnancy management options. 4th ed., Philadelphia, Elsevier Saunders; 2011: 1091-1092.
3. Carey JC, Gibbs RS. Preterm labor and delivery. In: Gibbs RS, Karlan BY, Haney AF, Nygaard IE, editors. Danforth's Obstetrics and Gynecology. 10th ed., Philadelphia, Walters Klumer Lippincott Wilkim's, & Wikins; 2008: 188.
4. Caughey AB, Robinson JN, Norwitz ER. Contemporary Diagnosis and Management of Preterm Premature Rupture of Membranes. Rev Obstet Gynecol. 2008;1(1):11-22.
5. Kim YH, Park YW, Kwon HS, Kwon JY, Kim BJ. Vaginal fluid B-human chorionic gonadotropin level in the diagnosis of premature rupture of membranes. Acta Obstet Gynecol Scand. 2005;84(8):802-805.
6. Kale E, Kuyumcuoglu U, Kale A, Guzel AI. Canoruc N. A new and practical aspartate aminotransferase test in vaginal washing fluid for the detection of preterm premature rupture of membranes. Fetal Diagn Ther. 2008;24(4):425-428.
7. Eriksen NL, Parisi VM, Daoust S, Flamm B, Grite TJ, Cox SM. Fetal fibronectin: a method for detecting the presence of amniotic fluid. Obstet Gynecol. 1992;80:451-454.
8. Kishida T, Yamada H, Furuta L, Kobayashi N, Hirayama EK, Ebina Y, et al. Persistent detection of alpha-fetoprotein in the vagina without overt preterm premature rupture of the membranes. Clinical and chemical characterizations. Fetal Diagn Ther. 2001;16(5):259-264.
9. Martinez de Tejada B, Boulvain M, Dumps P, Bischof P, Meisser A, Irion O. Can we improve the diagnosis of rupture of membranes? The value of insulin – like growth factor binding protein -1. Brit J Obstet Gynecol. 2006;113(9):1096-1099.
10. Kale A, Kale E, Akdeniz N. Kuyumcuoglu U, Canoruc N. proposal of a diagnostic test for the detection of preterm premature of membranes based on the determination of thyroid hormones in vaginal washing fluid. Fetal Diagn Ther. 2007;22(5):330-334.
11. Mangano B, Diani F, Faccini G, Zatti N, Zardini E. proposal of new test for diagnosis of PPROM based on determination of hCG in the washing fluid of the posterior vaginal fornix. Minerva Ginecol. 2000;52(5):185-188.
12. Ni YC, Jia WX, Yi WM, Feng LH, Yu LZ. Practicability of using vaginal fluid markers in detecting premature rupture of membranes. Ann Clin Biochem. 2003;40(5):542-545.
13. Esim E, Turan C, Unal O, Dansuk R, Cengizglu B. Diagnosis of premature rupture of membranes by identification of β-hCG in vaginal washing fluid. Eur J Obstet Gynecol Reprod Biol. 2003;107(1):37-40.
14. Cooper AL, Vermillion ST, Soper DE. Qualitative human chorionic gonadotropin testing of cervicovaginal washings for the detection of preterm premature rupture of membranes. Am J Obstet Gynecol. 2004;191(2):593-597.
15. Karimian NS, Jafari E, Amiri Moghadam HR, Majd HA, Mortazavi M. Comparing the diagnostic power of qualitative and quantitative measurements of β-hCG in cervicovaginal washing - fluid for diagnosis of PPROM. J Repord Infertil. 2007;8(1):30-37.
16. Anai T, Tanaka Y, Hirota Y, Miyakawa I. Vaginal fluid hCG levels for detecting premature rupture of membranes. Obstet Gynecol. 1997;89:261-264.

Authors Contribution:

Farideh Movahed and Khadijeh Elmizadeh designed the study and also revised the manuscript. Akram Choopani performed the study and collected data. Analysis and interpretation of data was done by Amir Javadi.